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Research Article

Total phenolic content and antioxidant activity of Water Hyacinth (*Eichhornia crassipes*) leaf extracts from different harvesting locations in South Sumatra

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Abstract: Oxidative stress is a condition where free radical levels exceed those of antioxidants. Thus, the body requires exogenous antioxidants, which can be obtained through functional foods or supplements. Differences in the nutrient, secondary metabolite content, and biological activity of medicinal plants may be influenced by environmental factors. Therefore, this study aimed to investigate the phenolic content and antioxidant activity of water hyacinth (*Eichhornia crassipes*) leaf extracts from different harvesting locations in South Sumatra. The results show that different harvesting locations affected the yields (17.62% – 26.59%), total phenolic contents (14.68 mg GAE/g – 34.10 mg GAE/g), and antioxidant activities (IC_{50} 176.10 µg/mL – IC_{50} 257.02 µg/mL) of the water hyacinth leaf extracts. The highest phenolic content was observed in the extract from Lubuklinggau, and this extract also exhibited more effective antioxidant activity compared to the Banyuasin and Palembang extracts. The asymmetric and symmetric stretching of the polymeric hydroxyl group and hydrogen bonding, which are characteristic features of phenolic compounds, were observed. This information suggests that water hyacinth leaves extract can be used as an alternative natural antioxidant agent for food supplements or functional food products.

INTRODUCTION

Reactive oxygen species (ROS) and other free radicals are continuously generated in the human body. When free radical levels exceed those of antioxidants, oxidative stress occurs. This condition is linked to several chronic, low-grade inflammatory diseases, such as insulin resistance in type-2 diabetes, cardiovascular diseases, and age-related disorders (Chaudhary *et al.*, 2023). Antioxidants are compounds capable of neutralizing the damaging effects of free radicals (Kalogerakou & Antoniadou, 2024). Various sources of antioxidants have been utilized, but most still rely on terrestrial plants. However, the demand for natural antioxidants continues to increase along with the rising prevalence of degenerative diseases and consumers' preference for natural products. This situation calls for the exploration of new potential and

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sustainable antioxidant sources. One promising alternative source is aquatic plants, which are relatively abundant, fast-growing, and potentially rich in bioactive compounds.

Several aquatic plants have been reported to possess antioxidant activity, including nipa palm (*Nypa fruticans*), yellow velvet leaf (*Limnocharis flava*), and water lettuce (*Pistia stratiotes*) (Sudirman *et al.,* 2023; Sudirman *et al.,* 2024; Sudirman *et al.,* 2024). The antioxidant activity of these plants is generally associated with their bioactive compounds, such as steroids, terpenoids, phenols (Sudirman *et al.,* 2017), polyphenols and tannins (Sudirman *et al.,* 2023), as well as flavonoids (Sudirman *et al.,* 2023). The main bioactive compounds dominant in water lettuce (*Pistia stratiotes*) extract are saponins and flavonoids, with IC_{50} values ranging from 147 to 193 ppm (Sudirman *et al.,* 2017). Nevertheless, the potential of aquatic plants as natural antioxidant sources has been relatively underexplored, particularly those originating from Sumatra.

One aquatic plant with promising potential is water hyacinth (*Eichhornia crassipes* (Mart.) Solms). This floating species is commonly found in Asian waters, including Indonesia, and can grow rapidly in rivers, lakes, and ponds under various environmental conditions (Pratiwi & Andhikawati, 2021). Several studies have reported that water hyacinth contains secondary metabolites and bioactive compounds such as polyphenols, alkaloids, and glycosides (Eden *et al.,* 2023; Hossain *et al.,* 2019). The phenolic and sterol compounds detected in water hyacinth indicate its richness in bioactive constituents, which could be utilized for drug development while also supporting the preservation of aquatic microecosystems (Anusiya *et al.,* 2020). These compounds are known to play important roles in its biological activities, including antioxidant activity.

Several studies in Indonesia have reported the antioxidant activity of water hyacinth, such as those from Palembang (Hasanah *et al.,* 2016; Baehaki *et al.,* 2022), Surakarta (Dewi & Avif, 2023), and Yogyakarta (Eden *et al.,* 2023). These studies generally used ethanol extraction or solvent fractionation, with the leaf parts exhibiting the highest activity. However, these studies were limited to single sampling sites. In fact, secondary metabolite content and biological activity of plants are influenced by environmental conditions such as ecology and climate (Pant *et al.,* 2021; Yahia *et al.,* 2020), including polyphenol content (Manach *et al.,* 2004), as demonstrated by the variation in phenolic levels of Tithonia diversifolia from different locations in Brazil (Sampaio *et al.,* 2016). To date, comparative multi-location studies on *E. crassipes*, especially across cities or regencies in Sumatra, remain very limited.

This research represents the first study in South Sumatra to compare the phenolic content of water hyacinth leaves from different locations. A modified maceration method was used, which has previously been reported to increase yield and preserve the stability of phenolic compounds (Chew *et al.,* 2011; Sudirman, Wardana, *et al.,* 2024). Therefore, this study aimed to investigate the phenolic content of *E. crassipes* from different harvesting locations in South Sumatra and evaluate its antioxidant activity.

METHOD

Sample Collection and Preparation

Fresh *E. crassipes* leaves were collected from different locations in South Sumatra, such as Banyuasin Regency (2º59’43” S, 104º50’17” E), Palembang City (2º58’50” S, 104º47’7” E), and Lubuklinggau City (3º15’54” S, 102º54’37” E). The sample was then transported to the laboratory for cleaning. Specimens are deposited in the Microbiology and Biotechnology Laboratory of Fisheries Product Technology, Universitas Sriwijaya (FPT0020032024). Next, the sample was cut into small pieces and dried using a dehydrator at 45ºC for 24 hours. After the drying process, the sample was ground using a grinder (Microphyte Disintegrator B-One DM-120M) into a powder and kept for further analysis.

Extraction Procedure

The extraction process used the maceration method and was performed according to the previous methods (Chew *et al.*, 2011; Sudirman *et al.*, 2024). Briefly, 20 g of dry powder from each location were mixed with 200 mL of 70% ethanol (ethanol in water, v/v) in an Erlenmeyer flask. The mixture was maintained at 30°C with stirring at 100 rpm using a magnetic stirrer for 2 hours. After 2 hours of maceration, the filtrate and residue were separated using filter paper (Whatman No. 42). The residue was then re-extracted with fresh solvent under the same conditions, and the process was repeated for a total of five extractions. Following extraction, the filtrates were combined in a new collection tube, and the solvent was removed using a rotary vacuum evaporator (Biobase RE-301) at 40°C to obtain a concentrated paste, which was subsequently freeze-dried (Biobase BK-FD10S). The yield of dry extract was calculated according to Formula (1).

$$\text{Yield (\%)} = \frac{\text{dry extract (g)}}{\text{dry sample (g)}} \text{ x } 100\% \quad (1)$$

Total Phenolic Content Analysis

The total phenolic content of water hyacinth leaf extracts was assessed using the Folin-Ciocalteu method, following the procedure outlined in a previous study (Chandra *et al.*, 2014; Sudirman, Herpandi, *et al.*, 2024). Briefly, 0.2 mL of each extract (10 mg/mL) was mixed with Folin-Ciocalteu's phenol reagent in a reaction tube and allowed to react for 5 minutes. After the reaction, 8% sodium carbonate (Na_2CO_3) was added, and the total volume was adjusted to 3 mL with distilled water (dH_2O). The mixture was then incubated in the dark for 30 minutes. After incubation, the supernatant was separated by centrifugation at 3,000 rpm for 30 minutes. The absorbance of the supernatant was measured at 750 nm using a spectrophotometer (Genesys 150, ThermoScientific, Massachusetts, USA). Gallic acid was used as the standard to quantify the total phenolic content, which was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample (mg GAE/g sample) according to Formula (2).

$$\text{Total phenolic content } \left(\frac{\text{mg GAE}}{\text{g sample}}\right) = X \frac{\text{Extract (mL)}}{\text{sample (g)}} \quad (2)$$

Whereas: X is the concentration of gallic acid (GA) from standard curve (mg GA/mL).

Antioxidant Activity Analysis

The antioxidant activity of water hyacinth leaf extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, following the procedures outlined in previous studies (Chew *et al.*, 2011; Sudirman *et al.*, 2024). Briefly, each extract was dissolved in ethanol to create a series of concentrations (0–1,000 µg/mL). Then, 1 mL of each sample solution at the specified concentrations was mixed with 1 mL of 0.2 mM DPPH in a reaction tube. The mixture was incubated at 37ºC for 30 minutes. The absorbance was immediately measured at 517 nm using a UV-Vis spectrophotometer. The antioxidant activity was expressed as the sample's ability to inhibit the DPPH radical and calculated according to Formula (3) and the half-maximal inhibitory concentration (IC_{50}) was calculated according to Formula (4).

$$\text{Percentage of inhibition (\%)} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \text{ x } 100\% \quad (3)$$

Whereas

Abs_{blank}=Absorbance at 517 without sample; Abs_{sample}=Absorbance at 517 with sample.

$$IC_{50} \text{ value } \left(\frac{\text{mg}}{\text{mL}}\right) = \frac{y - b}{a} \quad (4)$$

Whereas: y is 50% inhibition; a is the slope from the linear curve; b is the intercept from the linear curve.

Functional Group Analysis

The data are expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (one-way ANOVA), followed by Duncan's Multiple Range test (DMRT). A *p*-value of $p<0.05$ was considered statistically significant. Statistical analysis was conducted using SPSS version 22.0 (IBM Corporation).

Data Analysis

The data are expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (one-way ANOVA), followed by Duncan's Multiple Range test (DMRT). A *p*-value of $p<0.05$ was considered statistically significant. Statistical analysis was conducted using SPSS version 22.0 (IBM Corporation).

RESULTS

Extraction Yield

The extraction yield of *E. crassipes* leaf from different harvest locations is shown in Figure 1. The yield of the Banyuasin extract (26.59%) is significantly ($p<0.05$) higher compared to the Lubuklinggau extract (17.62%), and there is no significant ($p>0.05$) difference compared to the Palembang extract (22.79%).

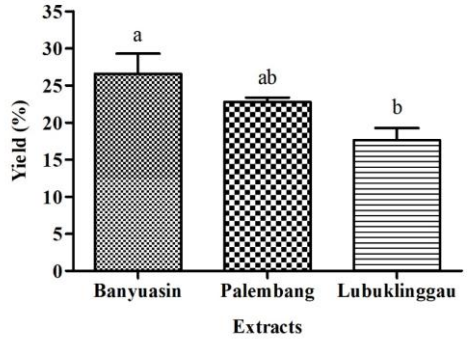


Figure 1. The yield of extraction of *E. crassipes* leaf from different harvesting locations. Data are shown as mean ± SD ($n=3$). The values with different letters (a-b) represent significantly different ($p<0.05$) as analyzed by Duncan's multiple range test.

Total Phenolic Content

The total phenolic content (TPC) of water hyacinth leaf extracts from different harvesting locations is shown in Figure 2. The Lubuklinggau extract (34.10 mg GAE/g dry sample) has a significantly ($p<0.05$) higher TPC compared to the Banyuasin extract (14.68 mg GAE/g dry sample) and the Palembang extract (18.32 mg GAE/g dry sample).

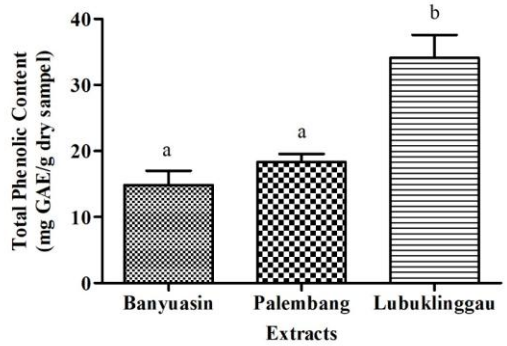


Figure 2. The total phenolic content of *E. crassipes* leaf extracts from different harvesting locations. Data are shown as mean ± SD ($n=3$). The values with different letters (a-b) represent significantly different ($p<0.05$) as analyzed by Duncan's multiple range test.

Antioxidant Activity

The antioxidant activity of water hyacinth leaf extracts from different harvesting locations is shown in Figure 3. The antioxidant activity of the extract is expressed as half-maximal inhibitory concentration (IC_{50}). The Lubuklinggau extract significantly ($p<0.05$) exhibits antioxidant activity compared to the Banyuasin and Palembang extracts, with IC_{50} values of 176.10 µg/mL, 261.42 µg/mL, and 257.02 µg/mL, respectively.

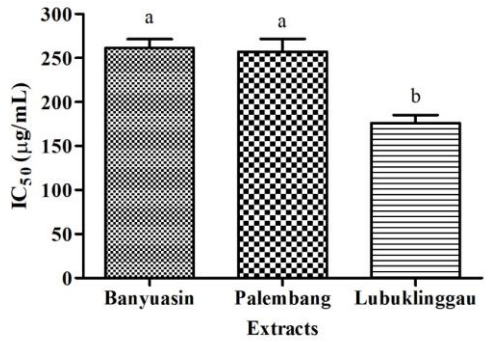


Figure 3. The antioxidant activity of *E. crassipes* leaf extracts from different harvesting locations. Data are shown as mean ± SD (n=3). The values with different letters (a-b) represent significantly different ($p<0.05$) as analyzed by Duncan's multiple range test.

Function Groups

The functional groups of phenolic compounds from water hyacinth leaf extracts are shown in Figure 4. Based on Figure 4, the spectra peaks of phenolic compound in the water hyacinth extract were observed at 3274.82 cm^{-1}, 1600.45 cm^{-1}, and 1268.54 cm^{-1} for the Lubuklinggau extract, 3366.43 cm^{-1} and 1596.11 cm^{-1} for the Banyuasin extract, as well as 3276.51 cm^{-1} and 1604.11 cm^{-1} for the Palembang extract as analyzed by FTIR spectroscopy.

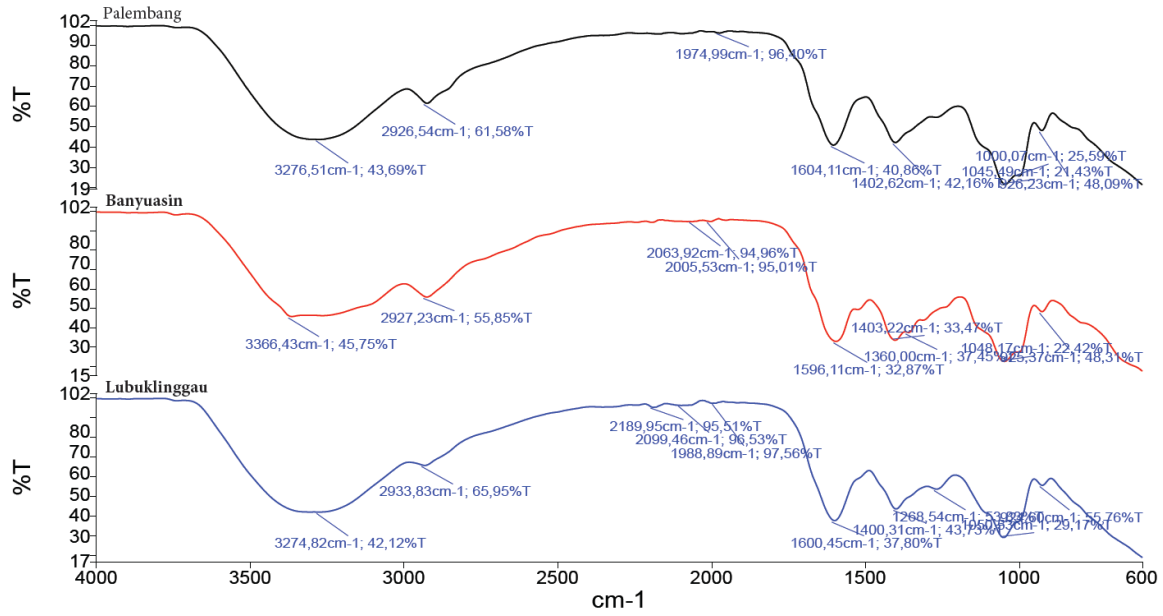


Figure 4. FTIR spectrum peaks of *E. crassipes* leaf extracts from different harvesting locations.

DISCUSSION and CONCLUSION

In this study, the phenolic compound was successfully extracted from the *E. crassipes* leaf at different harvesting locations in South Sumatera (Indonesia). The extraction yield of the water hyacinth leaf ranged from 17.62% to 26.59% (Figure 1). Previous studies have reported various extraction yields of water hyacinth leaves, including 56.73% using 70% ethanol (Putri *et al.*,

2023); 2.68% (w/w) crude extract using 96% ethanol, followed by fractionation with n-hexane (30.94%), ethyl acetate (8.74%), and water (60.32%) (Hasanah *et al.*, 2016); and 8.9% (w/w) crude methanol extract (Ukhty, 2021). Apart from the type of solvent used (Hasanah *et al.*, 2016), other parameters such as solvent concentration and extraction time also affect the extraction yield of water hyacinth (Putri *et al.*, 2023). Moreover, geographical variation can influence yield, as demonstrated in *Pterospartum tridentatum* stems, which showed extraction yields ranging from 14.8% to 16.8% depending on the harvest location (Coelho *et al.*, 2011). These findings highlight that extraction yields can differ according to environmental conditions and the site of plant collection. The relatively higher yield obtained from Banyuasin samples may reflect a greater abundance of phenolic compounds, potentially resulting in stronger antioxidant activity.

The phenolic content of *E. crassipes* leaves varied considerably across harvesting locations, ranging from 14.68 to 34.10 mg GAE/g dry sample (Figure 2). These values are higher than those reported by (Dewi & Avif, 2023) (5.40 mg GAE/g) from Surakarta. Similar patterns have been observed in other species, such as Croton macrostachyus, which showed 8.02–15.57 mg GAE/g across different sites in Ethiopia (Ayele *et al.*, 2022), and Tithonia diversifolia from Brazil, which also exhibited location-dependent variation (Sampaio *et al.*, 2016). This indicates that phenolic accumulation in water hyacinth can vary markedly among regions. Differences in nutrient and secondary metabolite content in plants are known to be influenced by environmental factors, such as ecological and climatic conditions (Pant *et al.*, 2021), which have a major effect on polyphenol content (Manach *et al.*, 2004). These findings suggest that the higher phenolic content observed in the Lubuklinggau samples may be linked to favorable local environmental conditions, which could potentially enhance the antioxidant activity of the extracts. Therefore, selecting appropriate harvesting sites could be crucial for maximizing the functional properties of water hyacinth as a natural antioxidant source.

The antioxidant activity of *E. crassipes* leaves varied across sampling locations, with IC_{50} values ranging from 176.10 to 257.02 µg/mL (Figure 3), indicating that location differences influence the bioactivity potential of the plant. This finding aligns with that of Yahia *et al.* (2020), who reported variations in the antioxidant activity of Ziziphus lotus leaf extracts from different locations in Tunisia (IC_{50} 16.60–18.27 µg/mL), as well as Ayele *et al.*(2022), who found differences in the antioxidant activity of Croton macrostachyus root extracts from various locations in Ethiopia (3.53–6.38 mg AAE/g).

Compared to previous studies on *E. crassipes*, the antioxidant activity (IC_{50}) observed in this study falls within the moderate to weak range, as several studies have reported stronger activity using the DPPH assay, such as 48.64 µg/mL from Boyolali (Dewi & Avif, 2023) and 164 µg/mL from Palembang (Hasanah *et al.*, 2016). In addition, Eden *et al.* (2023) reported stronger antioxidant activity of *E. crassipes* leaves using the ABTS and FRAP assays, with values of 42.91 µg/mL and 381.98 µg/mL, respectively. The IC_{50} values of the extracts in this study were also higher (indicating weaker activity) than the positive control ascorbic acid, which showed an IC_{50} of 50.71 µg/mL (Hasanah *et al.*, 2016). Among the samples tested, the Lubuklinggau extract exhibited the strongest radical scavenging activity (IC_{50} 176.10 µg/mL), which was consistent with its highest total phenolic content (TPC) of 34.10 mg GAE/g. A positive correlation between TPC and antioxidant activity has also been reported by (Omara *et al.*, 2021). These results suggest that the variation in antioxidant activity across locations is likely influenced by differences in phenolic content, which are shaped by local environmental factors such as water nutrient availability, light intensity, and abiotic stress factors known to affect the biosynthesis of secondary metabolites (Costa *et al.*, 2022). This highlights the need to consider location-based variability and establish raw material standardization when evaluating the bioactivity of water hyacinth.

The interpretation of the FTIR spectrum peaks related to functional groups is based on previous studies (Nandiyanto *et al.*, 2019; Wongsa *et al.*, 2022). The peaks indicate the stretching of the

polymeric hydroxyl group (O-H) and hydrogen-bonded stretching at 3274.82 cm^{-1} (Lubuklinggau extract), 3366.43 cm^{-1} (Banyuasin extract), and 3276.51 cm^{-1} (Palembang extract). Figure 4 also shows aromatic C=C stretching at 1600.45 cm^{-1} (Lubuklinggau extract), 1596.11 cm^{-1} (Banyuasin extract), and 1604.11 cm^{-1} (Palembang extract). Additionally, C–O stretching is detected in the Lubuklinggau extract at 1268.54 cm^{-1}. The region between 3400 and 3200 cm^{-1} is associated with the asymmetric and symmetric stretching of the polymeric hydroxyl (O-H) group and hydrogen bonding, which is a characteristic feature of phenolic compounds. Phenolic C–O stretching was observed at approximately 1200 cm^{-1}. The region between 1400 cm^{-1} and 900 cm^{-1} is often referred to as the fingerprint region, due to the numerous characteristic single bands of low intensity, which correspond to specific functional groups (Wongsa *et al.*, 2022).

Overall, phenolic compounds were successfully extracted from the *E. crassipes* leaf harvested from different locations in South Sumatra. The different harvesting locations affected the yield, total phenolic content, and antioxidant activity of the water hyacinth leaf extract. The highest phenolic content was observed in the extract from Lubuklinggau, and this extract also exhibited more effective antioxidant activity compared to the Banyuasin and Palembang extracts. The asymmetric and symmetric stretching of the polymeric hydroxyl group and hydrogen bonding, which are characteristic features of phenolic compounds, were observed. Although the results of this study indicate that water hyacinth leaves contain phenolic compounds with antioxidant potential, the antioxidant activity analysis conducted was limited to the DPPH assay. A single method is not sufficient to comprehensively represent antioxidant capacity. A more comprehensive evaluation could be achieved by including other methods, such as FRAP or CUPRAC, which assess antioxidant mechanisms from different perspectives (Hasanah *et al.*, 2016) and analyze the main bioactive compounds in water hyacinth, such as phenolics, flavonoids, and sterols, to understand their role in antioxidant activity. Therefore, further studies involving various antioxidant assays and positive control comparisons are needed to validate their effectiveness and safety before proposing their development as ingredients for dietary supplements or functional food products.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-maill upon request.

Authorship Contribution Statement

R.U.: Analysis and/or Interpretation. **P.A.P.:** Data Collection and/or Processing. **M.J.:** Analysis and/or Interpretation. **S.S.:** Conception, Supervision and Writing.

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